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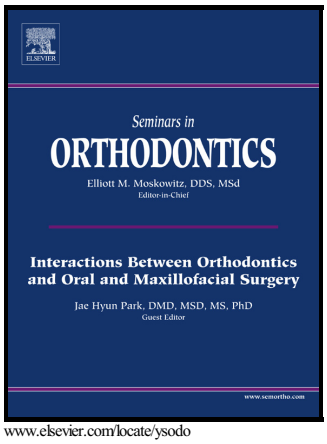
Suveer Sachdeva, Martyn T. Cobourne



www.elsevier.com/locate/ysodo

PII: S1073-8746(17)30038-5
DOI: http://dx.doi.org/10.1053/j.sodo.2017.07.004
Reference: YSODO510

To appear in: *Seminars in Orthodontics*

Cite this article as: Suveer Sachdeva and Martyn T. Cobourne, Dental mesenchymal stem cell research – how much will it translate to clinical orthodontics?, *Seminars in Orthodontics,* http://dx.doi.org/10.1053/j.sodo.2017.07.004

Dental mesenchymal stem cell research – how much will it translate to clinical orthodontics?

Suveer Sachdeva

Department of Craniofacial Development and Stem Cell Biology

King's College London Dental Institute

Floor 27, Guy's Hospital

London SE1 9RT, UK

Martyn T. Cobourne

Department of Orthodontics

King's College London Dental Institute

Floor 27, Guy's Hospital

London SE1 9RT, UK

Abstract

Oral mesenchymal stem cell populations have been identified in association with the mucosal tissues and both deciduous and permanent teeth in humans. These cells demonstrate in vitro characteristics that include the expression of specific markers, self-renewal and the capacity to differentiate into multiple cell types. The relative accessibility of these cell populations means that they may represent a source of stem cells with great potential for use in tissue regeneration. Significant research is now being carried out to further identify the origins, properties and potential applications of these cells and it is likely that they will have a significant impact on clinical dentistry over the coming decades. Here we review current knowledge relating to the biology of oral mesenchymal stem cells, discuss their wider potential applications within regenerative dentistry and speculate on their future role in clinical orthodontics.

Introduction

Stem cells are undifferentiated cells found in multicellular organisms that have the capacity to both self-renew or differentiate into multiple different cell types. Archetypal totipotent stem cells can ultimately differentiate into all cells within the body as well as the extra-embryonic cells and are only found in the very early embryo. Pluripotent stem cells can differentiate into all cell types within the body but not extra-embryonic cells and are found within the inner cell mass of the blastocyst. Multipotent mesenchymal stem cells (MSCs) are found in the tissues of mature (or adult) animals and have the dual generic properties of being able to differentiate into multiple cell types and demonstrate immunomodulatory activity; however, they are more limited in the type of cell that they can differentiate into and display variation in the extent to which they can form different cell types. These cells are often referred to as adult stem cells and include those found in the haematopoietic system, which differentiate into different components of the blood system during normal function. MSCs are found in a variety of body tissues and organs, and essentially function to replace specific cell types within these regions during normal tissue homeostasis or repair. Archetypal sources include those derived from bone marrow, adipose tissue and the umbilical cord. The attraction of MSCs within medicine is that they can be harvested from the body, isolated, expanded and programmed to differentiate into a specific cell type, before being returned to the host. This cell type might be organ-specific but also, and potentially more useful, non-organ-specific. Although totipotent and pluripotent stem cells can differentiate into a larger repertoire of cell types, the ethical and immunological implications of using these cells makes them less attractive. In relatively recent times, multiple different types of MSC have been identified in the oral cavity and their relative accessibility for harvest has made them a useful potential source of cells with possible therapeutic applications.

Oral mesenchymal stem cells

A number of MSC populations exist in tissues of the oral cavity and these have generally been named according to their anatomical origin (Figure 1) [1]. Dental mesenchymal stem cells (DMSCs) are broadly defined as those MSCs with the ability to form a dentine-pulp complex in vivo [2] and the first of these were identified at the start of the new millennium in the pulp of extracted third permanent molars and named (postnatal) Dental Pulp Stem Cells (DPSCs) [3]. Perhaps not surprisingly, DMSCs have also been identified in the pulp of deciduous teeth and termed Stem cells from Human Exfoliated Deciduous teeth or SHED cells [4]. Further DMSC populations have been isolated from the apical foramen of third molars that have not completed root development, named Stem Cells from the Apical dental Papilla (SCAP) [5, 6] and from the dental follicle as Dental Follicle Precursor stem Cells (DFPC) [7, 8]. A group of non-dental MSCs have also been identified in the periodontal ligament, Periodontal Ligament Stem Cells (PDLSCs) [9] and also a number derived from sub-epithelial layers of the oral mucosa and gingiva [10] that have a number of terms and include Gingiva-derived Mesenchymal Stem Cells (GMSC) [11], Gingival Tissue-derived Mesenchymal Stem Cells (GT-MSC) [12], Human Oral Mucosa Stem Cells (hOM-SC) [13] and Oral Mucosa Lamina Propria Progenitor Cells (OMLP-PC) [14].

Each of these MSC populations shares the capacity for self-renewal, but they have specific gene expression profiles and differ in their ability to differentiate into different cell lineages [10, 15]. They all have adipogenic, chondrogenic, osteogenic and neurogenic multipotentiality [5, 6, 16-22] and consistent with their anatomical location DFPC, SCAP and SHED cells can all differentiate into odontoblast-like cells and in the case of DFPC and SCAP cells, produce a robust dentine-pulp-like complex and in the functioning tooth are almost certainly involved in generating coronal and root dentine, respectively [18, 23]. Complete pulp regeneration has been achieved harnessing DPSCs transplanted with stromal cell-

derived factor 1 (SDF1) in a collagen scaffold using a canine model [24]. SHED cells can produce dentine-pulp-like tissue [4]; whilst PDLSC and DFPC cells are both able to form ectopic tissue with periodontal ligament-like characteristics and cementum-like or cementum matrix, respectively. Indeed, PDLSC cells can recapitulate a periodontal ligament-like structure with cementoblasts and appropriately orientated collagen fibres [9].

Origins of oral mesenchymal stem cells

In the last two decades, significant progress has been made in our understanding of how tooth development is regulated at the molecular level and much of this work has been conducted using the mouse molar as a developmental model. In more recent years, the murine incisor has become an essential model to investigate the biology of MSCs, largely because this tooth has the capacity to continuously grow throughout the life of the animal and therefore contains specific populations of mesenchymal and indeed, epithelial stem cells [25]. It has been shown that there are multiple sources of DPSCs in the murine incisor, which can differentiate into pulp and odontoblast cells. Sophisticated genetic labelling studies have demonstrated that a significant proportion of MSCs in the tooth are derived from peripheral nerve-associated glia [26]; whilst perivascular cells (pericytes) situated on the abluminal surface of endothelial cells within the pulpal microvasculature also contribute to a proportion of the odontoblast population during growth and repair [27, 28]. In the murine incisor, further lineage-tracing experiments have identified the neurovascular bundle as a MSC niche, with sensory nerves able to secrete the Sonic hedgehog signaling protein and activate *Gli1* expression in periarterial cells [29]. Interestingly, this investigation has provided further insight into the identification of more elusive *in vivo* (as opposed to *in vitro*) MSC markers and their relevance to stem cell function. *Gli1*-positive periarterial cells support incisor homeostasis and give rise to the entire population of MSCs *in vitro,* but do not express classic *in vitro*

MSC markers. In contrast, pericytes are a *NG2*-positive MSC sub-population that do express conventional *in vitro* MSC markers, but these cells seem to contribute mainly to injury repair rather than homeostasis [29].

Clinical applications of oral mesenchymal stem cells

The key potential applications for oral MSCs are related to their inherent functional capacity to differentiate into specific cell types and generate adult tissues. Here, we will focus primarily on the regeneration of dental tissues but in the future, these cells might also be used to regenerate non-dental tissues. In global terms, both dental caries and periodontal disease represent the commonest causes of damage to the dentition and supporting tissues, and ultimately premature tooth loss. The use of oral MSCs to repair or replace damaged pulpal and periodontal tissues is currently an area of significant research activity.

Tooth pulp restoration

The use of DPSCs to regenerate dental pulp tissue is an obvious potential therapeutic goal in dentistry. Pulp regeneration and the formation of secondary dentine is functionally close to the inherent role of these cells in the natural tooth and dental caries is a significant global health problem. The use of pulpotomy, or pulpectomy and root canal treatment to restore and preserve teeth following a pulpitis is time-consuming and expensive. Moreover, it can be associated with varying degrees of failure, including postoperative pain, periapical infection, tooth fracture and ultimately, dental extraction. Some recent progress has been made in this field, with the demonstration of complete pulp regeneration in a canine pulpitis model following the transplantation of autologous DPSCs supplemented with SDF1 in a collagen scaffold [24]. Human DPSCs have been shown to have improved regenerative potential when isolated using granulocyte colony-stimulating factor-induced mobilization (mobilized dental

pulp stem cells; MDPSC) [30] and autologous transplantation of these cells has recently been reported in a Japanese pilot clinical study. A sample of five subjects with irreversible pulpitis and pulpectomy were monitored for twenty-four weeks following transplantation of MDPSCs using electric pulp-testing, magnetic resonance imaging (MRI), conventional dental radiography and cone-beam computerized tomographic (CBCT) imaging. There were no adverse events or toxicity reported in any of the patients and a positive vitality-response in the majority, which was accompanied by a similar level of signal intensity of the regenerated tissue when compared to normal pulp and evidence of functional dentine formation [31]. This type of therapy has absolute future potential to provide an alternative to traditional forms of root canal treatment.

Periodontal ligament regeneration

Periodontal disease represents another significant cause of tooth loss characterised by an irreversible loss of attachment associated with the periodontal tissues and alveolar bone. The periodontal ligament is a specialized connective tissue that includes both hard and soft tissues, which makes regeneration challenging. The concept of periodontal ligament regeneration is not new and a variety of techniques have been described, including the use of surgery, guided tissue regeneration, bone fillers and growth factors. However, epithelial migration into the area of regeneration and formation of a long junctional epithelium is a significant problem and long-term regeneration remains elusive [32].

The identification and subsequent characterization of PDLSCs has encouraged the concept of stem cell-based periodontal ligament regeneration [33]. These cells can form periodontal ligament-specific structures when transplanted into ectopic locations in mouse models [9] and there are reports of periodontal regeneration following the transfer of autologous PDLSCs into surgically-created periodontal defects in both Miniature pig and

Beagle models [34-37]. More recently, there have been reports of enhanced periodontal regeneration in surgically-created periodontal defects in rat models following the transplantation of PDLSCs as multi-layered pellets or on an amniotic membrane [38, 39]. However, the periodontal ligament has a complex architecture and significant challenges remain before this connective tissue can be regenerated routinely.

Whole tooth bioengineering

A major goal of stem cell-based research in dentistry has always been tooth replacement through bioengineering. Our knowledge of the epithelial-mesenchymal interactions that underpin normal tooth development and more recent advances in both stem cell biology and tissue engineering have provided a theoretical basis for achieving this goal [40] . However, many obvious difficulties do exist, not least the significant time-length required to generate and erupt a human tooth, aesthetic considerations that might be difficult to regulate and control biologically and the need to reproduce and establish appropriate long-term physiological function.

The essential strategy for generating a bioengineered tooth involves isolating a population of (stem) cells that can mimic the reciprocal epithelial-mesenchymal interactions required to generate a tooth and providing them with a suitable in vitro environment to develop into a tooth primordium. This primordium is then transplanted into the host jaw, either as a tooth germ or fully functional unit (Figure 2) [41]. Experiments with disassociated and cultured embryonic tooth primordia followed in rodent models have shown very clearly that tooth development and eruption can occur in an adult jaw following their transplantation [41-43]. The main current challenge is obtaining suitable sources of adult epithelial and mesenchymal cells that are capable of inducing tooth formation that can be generated and cultured in sufficient numbers to allow transplantation and subsequent generation of a

bioengineered tooth. There has been some success in rodent models using embryonic tooth epithelium and adult bone marrow stromal cells in bioengineered tooth formation [44] and more recently, human adult epithelial cells combined with embryonic tooth-inducing mesenchyme [45].

Until recently, the concept of tooth bioengineering had been limited to rodent models; however, this technique has now been used in a Beagle dog model [46]. This investigation has demonstrated that functional tooth development can be achieved in higher mammals using epithelial and mesenchymal cells; not only derived from embryos, but also from postnatal tooth germs. This represents an exciting potential model because it offers the possibility of autologous transplantation of engineered teeth in humans, which avoids the problems of immune rejection [46].

Orthodontic-specific applications of oral MSCs

There are many potential applications for the use of oral MSCs in the orthodontic clinic although it is likely that these will not become routine for the foreseeable future. Some obvious examples include the potential for bone generation in the craniofacial region, including the alveolus [47]; however, more specific applications also exist.

Orthodontic tooth movement

Orthodontic tooth movement (OTM) is fundamental to all types of orthodontic treatment and the tissue changes that occur within the periodontium following the application of orthodontic force have been studied for many years. It is generally accepted that OTM occurs through a sterile inflammatory reaction that is instituted within the periodontal ligament and results in alveolar bone resorption in regions of compression, deposition in those areas under tension and significant remodelling of the periodontal ligament. However, there is currently

relatively little known about the response and role of PDLSCs following the application of mechanical force. Using CD90 as a marker for these stem cells it has recently been demonstrated in a rodent model that their numbers increase in regions of compression, whilst their expression of collagen-1 is reduced. Interestingly, after the withdrawal of orthodontic force, PDLSCs accumulated in degradation regions and increased their levels of collagen-1 expression during early relapse. Therefore, PDLSCs respond to the application of orthodontic force with suppressed collagen expression, which is subsequently regained after force withdrawal [48]. The manipulation of PDLSC biology to accelerate OTM and perhaps more importantly, promote stability of tooth position is therefore an intriguing possibility, albeit one that may be some way in the future.

External root resorption

A common side-effect of OTM is external apical root resorption, which causes a loss of root cementum and dentine that in severe cases, can significantly impact on longevity of the affected tooth or teeth. No therapeutic strategies are currently available to manage this condition, either during or following OTM other than the cessation of treatment. However, stem cell-mediated regeneration of cementum offers a potential solution and both the range and anatomical location of DMSCs that have been identified to date offers some promise that a suitable source can be identified. Although this concept remains in its infancy, it has recently been demonstrated that PDLSCs isolated through outgrowth were able to form cellular cementum-like hard tissue containing embedded osteocalcin-embedded cells following in vivo transplantation [49].

Dental epithelial stem cells

The enamel of the tooth crown lacks any capacity to regenerate in human teeth and so it is perhaps not surprising, that epithelial stem cells have not been identified in either the deciduous or permanent dentitions. However, self-renewing epithelial stem cells have been identified in the oral mucosa [50] and their ability to form sheets of stratified epithelium has been used to promote corneal repair [51]. Interestingly, adult human gingival epithelial cells are also capable of supporting tooth development when recombined and cultured with murine cap stage embryonic molar mesenchyme [45].

Future perspectives

Oral MSCs provide a useful and accessible source of stem cells with potential for use in a range of regenerative therapies for use in dentistry, including orthodontics. However, significant challenges remain in our understanding of the biology underlying these cells. The obvious challenge is refining our ability to control what they do in vivo and this will involve a range of subject areas, including cell biology and tissue regeneration. However, we also need to understand how these cells can be efficiently identified, harvested, expanded and crucially, reproduce their *in vitro* characteristics *in vivo*. Progress is likely to move at a rapid pace.

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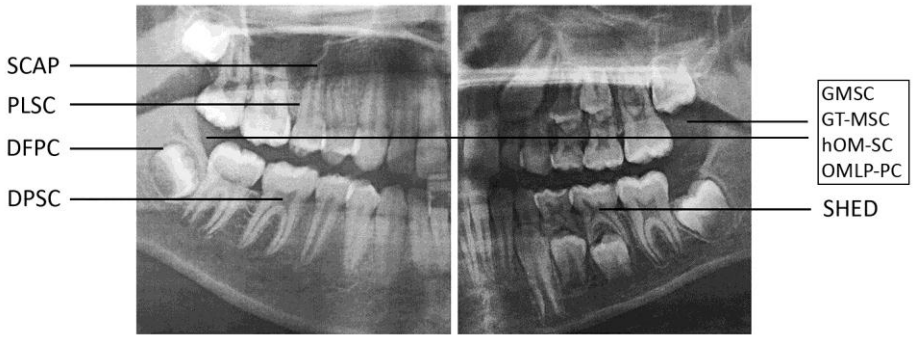
Figure legends

Figure 1

Sources of oral mesenchymal stem cells. Dental mesenchymal stem cells include Dental Pulp Stem Cells (DPSC); Stem Cells from the Apical dental Papilla (SCAP); Dental Follicle Precursor stem Cells (DFPC); Stem cells from Human Exfoliated Deciduous teeth (SHED). Non-dental mesenchymal stem cells include Periodontal Ligament Stem Cells (PDLSC) and those originating from the oral mucosa (boxed), which include Gingiva-derived Mesenchymal Stem Cells (GMSC), Gingival Tissue-derived Mesenchymal Stem Cells (GT-MSC), Human Oral Mucosa Stem Cells (hOM-SC) and Oral Mucosa Lamina Propria Progenitor Cells (OMLP-PC).

Figure 2

Fundamental strategies for biological tooth replacement. Epithelial and mesenchymal cell sources are isolated and recombined *in vitro* to induce formation of an early tooth germ. The tooth germ is either transplanted into the recipient jaw at the cap stage (left), which is followed by continued development and eruption into the oral cavity (functional tooth regeneration); or the tooth germ is allowed to continue development toward the formation of a bioengineered tooth unit, including a periodontal ligament and alveolar bone (right), which is then grafted into the host site (functional tooth replacement). Adapted from [52, 53].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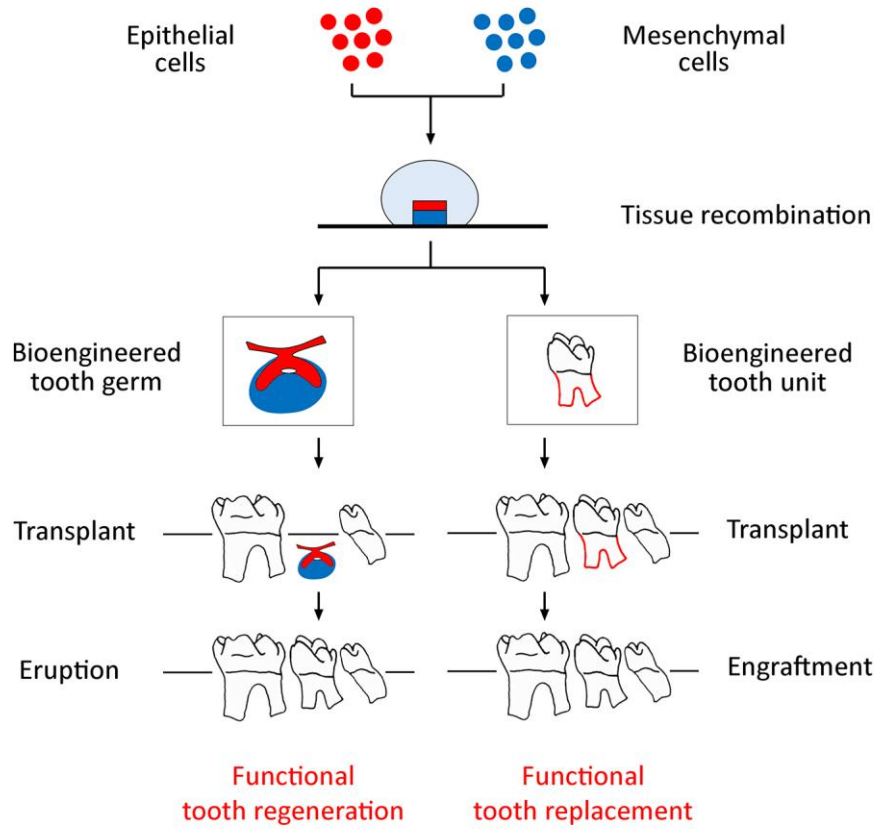
Epithelial cells
Mesenchymal cells
Tissue recombination
Bioengineered tooth germ
Bioengineered tooth unit
Transplant
Transplant
Eruption
Engraftment
Functional tooth regeneration
Functional tooth replacement